

## Gonadotrophin in the Urine of a Pregnant Indian Elephant— A Case Report

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(Plates I-II)

In 1963, at Takarazuka Zoo, Japan, a young female Indian elephant became pregnant, and in May, 1965, she gave birth to a very large stillborn calf (weighing 133.3 kg, male). The time of conception was problematical, but it was assumed as April or May of 1963, hence the gestation period may have been 24 or 25 months, a little longer than average.

Pregnancy diagnosis was attempted during the early and middle gestation period. For exploration, an urinary gonadotrophin was checked by the Friedman and Aschheim-Zondek tests on the whole urine samples collected twice in August 1963. Results showed apparently positive responses in both tests.

However, the samples collected in May and September, 1964, showed negative in the three tests, including a male frog (*Rana*) reaction which was subjected to the concentrated urine samples.

So, probably a gonadotrophic substance may have been excreted in urine of this elephant at some time of the early pregnancy, and this may be more like FSH than LH in its activity.

### INTRODUCTION

THE ZOO at Takarazuka, Hyogo, Japan, has maintained two Indian elephants for several years. In 1962, the male was 14 years old and the female 15 years old. Mating behavior was first observed in April 1962 and was frequently observed during the day and night. The female became pregnant and gave birth in May 1965 to an abnormally large, male stillborn calf in posterior presentation. It weighed 133.3 kg. The time of conception was problematical, but it was assumed that conception occurred in April or May, 1963. Hence, the gestation period was about 24 to 25 months, a little longer than average (Nalbandov, 1964; Parkes, 1956; Perry, 1953).

Pregnancy diagnosis was attempted during the early and middle stages of gestation period. Urine samples were examined for gonadotrophins by the Friedman, Aschheim-Zondek, and male frog tests. The Friedman and Aschheim-Zondek tests on whole urine collected twice in August 1963 were positive, but whole and concentrated urine samples collected in May and

September, 1964, were negative on these three tests.

Thus, it appeared that a gonadotrophic substance was excreted in this pregnant elephant's urine during the third to the fourth month of gestation, but was not present in the urine at the twelfth to thirteenth and the sixteenth to seventeenth month of a 24 to 25 months gestation period.

### MATERIALS AND METHODS

Urine samples were collected at three periods of time during the elephant's pregnancy; in the second and the fourth week of August, 1963; in the first week of May, 1964; and in the second week of September, 1964. As a control, urine from a 17-year-old non-pregnant female elephant, which had been raised with another female for years at the Hanshin Park Zoo, Nishinomiya, was collected the fourth week of August, 1963.

Urine samples were collected directly with a ladle (Pl. I, fig. 2) during urination. Each urine sample collected had a pH of 8.6 to 8.8. The urine was weakly acidified to pH 5.0 to 6.0 with acetic acid and filtered through both clean absorbent cotton, that had been washed in ethanol and ether and then dried, and paper. The urine was then washed with three volumes of ethyl ether in a large separatory funnel for about three

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minutes for the purpose of removing possible toxic substances and steroids. The washed urine was placed in a warm water bath of 37 C, for seven to eight minutes. While stirring with a glass rod, a stream of nitrogen gas was blown on the washed urine to drive off the small amount of ether that remained in it. The control urine was treated similarly.

A modified Friedman test was done. The urine sample for injection was separated into two equal portions. Each portion was injected into each of two Japanese white rabbits, one young and the other adult. Twenty-four hours before the first injection, the ovaries and uterus of each rabbit were examined by laparotomy. After confirming that no large, protruding hemorrhagic follicles or corpora hemorrhagica existed on the ovaries and that the uterus was normal, the first half-dose of urine was injected into the ear vein of each rabbit. Twenty-four hours later the second-half dose was given, and autopsy was done 48 hours after the first injection (Table 1). Thirty I.U. of human chorionic gonadotrophin (HCG) dissolved in saline solution was injected into another rabbit as a positive control.

The Aschheim-Zondek test used in this experiment was also modified. Each of five immature female rats, Wistar strain, weighing 29 gm

to 37 gm was given injections subcutaneously with urine, six times in three successive days, and an autopsy was performed 116 to 120 hours after the first injection (Table 2). Beside the findings of follicles or corpora hemorrhagica on the ovaries of each test rat, the weight of the ovaries of test and control rats were compared and recorded.

The urine concentrate was prepared by the addition of 1/20 amounts (v/v) of 20 percent acid-washed kaolin suspension to the urine, as an adsorbing agent, and processed by the method suggested either by Cutler (1949), and Bradbury *et al* (1949).

Each 500 ml of whole urine was finally concentrated to a 25 ml solution according to each method. The concentrated urine samples were used in the frog test, the Friedman test, and Aschheim-Zondek test.

The male *Rana nigromaculata* Hallowel (Japanese rana frog) was chosen as the test animal for the frog test. Two ml of the above concentrated solutions was injected into the dorsal lymph sac at each side of five frogs. Thirty I.U. or HCG dissolved in saline solution was injected into a frog for a positive control. The fluid in the cloaca was pipetted every 30 minutes for a two hour period and was examined under micro-

TABLE 1. RESULT OF THE FRIEDMAN TEST WITH THE WHOLE URINE OF A PREGNANT INDIAN ELEPHANT

No. of Urine Samples	No. and Body Weight of Rabbit (gm)	Ml. of Urine injected (i.v.)		Autopsy Findings in Ovaries (1) in Uterus (2)	Judgment
		1st	2nd		
1	1 (1340)	3.5	3.5	(1) 9 hemorrhagic follicles (2) enlarged & hyperemic	positive
	2 (2200)	6.0	6.0	(1) 11 hemorrhagic follicles (2) enlarged & hyperemic	positive
2	3 (1330)	3.3	3.3	(1) 8-9 hemorrhagic follicles (2) enlarged & hyperemic	positive
	4 (2740)	6.5	6.5	(1) 9-10 hemorrhagic follicles (2) enlarged & hyperemic	positive
control	5 (2620)	6.5	6.5	(1) many small follicles (2) small	negative

- (1) Samples: 1 — whole urine, second week, August, 1963  
 2 — whole urine, fourth week, August, 1963  
 control — whole urine, non-pregnant, fourth week, 1963

- (2) Time of injections:  
 1st inj.: at 0-hour, 2nd inj.: at 24-hour

- (3) Time of Autopsy: at 48-hour

scope. When examinations at 30, 60, 90, and 120 minutes showed no spermatozoa in the cloacal fluid, a "negative" report was given.

### RESULTS

Whole urine samples 1 and 2, of August 1963, were both positive on the Friedman test as indicated by the presence of hemorrhagic follicles on ovaries of the four test rabbits. The whole urine of the control female elephant was negative.

In the Aschheim-Zondek test using immature Wistar rats, weighing 29 gm to 37 gm, the positive reaction was weak with whole urine sample 1, but stronger with whole urine sample 2 (Table 2). The average weight increase of ovaries of test rats was 2.4 and 3.5 times, respectively, that of the control rat. The concentrated and whole urine samples collected in May and September, 1964, (sample 3 and 4) were negative in the frog test or the other three tests (Table 3).

Though urine samples 1 and 2, collected at the third or fourth month of gestation were positive, unfortunately, no further examinations were done in 1963 to follow the excretion pattern of gonadotrophic substance in urine of this elephant.

Thus it could be concluded that in the third to fourth month of gestation, some gonadotrophic substance was excreted in this elephant's urine, and it had disappeared by the twelfth to thirteenth month of gestation.

### DISCUSSION

The details of the behavioral observations throughout estrus, mating, and parturition of this elephant were made in 1966 (Koto and Fujimoto). Nalbandov (1964) noted that it has been reported that the elephant (as the mare) forms accessory corpora lutea, but only from about the end of the sixth to the ninth month of the 24 months gestation. He inferred that elephants secrete a gonadotrophic substance similar to the one produced by pregnant mares. So, there seemed to be some difference in time in the secretion or excretion of gonadotrophin, between the findings obtained by them, six to nine months, and by us, three to four months. However, the time of conception may probably be hard to judge in most pregnant elephants. Actually in the experiment recorded here, "mating behavior" was often observed over a long period of time, apparently even in the pregnant period. True or successful copulation, however, was only observed once at midnight by an attendant (June 16, 1962) and the mating was infertile.

TABLE 2. THE ASCHHEIM-ZONDEK (RAT) TEST ON URINE FROM A 3-4 MONTH PREGNANT INDIAN ELEPHANT

#### 1. Technic of Test

Day	Injection of Urine		
	Morning	Noon	Evening
1	1st	—	2nd
2	3rd	4th	5th
3	6th	—	—
6	Autopsy (at 116 to 120 hours after the 1st injection)		

#### 2. Results of Test

No. of rat	Volume of Urine per Injection (1st to 6th)	No. of Samples					
		1		2		control	
		Reaction	Judgment	Reaction	Judgment	Reaction	Judgment
1	0.3 ml each	I		I		0	
2	0.5 " "	II		II		0	
3	1.0 " "	I	pos.	II	pos.	0	neg.
4	1.5 " "	II		II		0	
5	2.0 " "	I		II		0	

(1) Reaction: 0 — very small follicles only

I — large follicles

II — hemorrhagic follicles

III — hemorrhagic corpora lutea

(2) Urine samples:

No. 1 — whole urine, second week of August, 1963

No. 2 — whole urine, fourth week of August, 1963

control — whole urine, non-pregnant elephant, fourth week of August, 1963



TABLE 3. SUMMARY OF RESULTS OF TESTS ON THE URINE OF A PREGNANT INDIAN ELEPHANT

Sample No.	Time in Gestation, urine collected	Urine	Test	Result	Controls
1	3-4 mons. (Aug., '63)	whole	Friedman Asch.-Zond.	pos. pos.	pos. (HCG) neg. (saline)
2	3-4 mons. (Aug., '63)	whole	Friedman Asch.-Zond.	pos. pos.	neg. (urine) neg. (urine)
3	12-13 mons. (May, '64)	concentrated by method (A)*	Frog (male)	neg.	pos. (HCG) neg. (saline)
4	16-17 mons. (Sept., '64)	1. whole	Friedman Asch.-Zond.	neg.	pos. (HCG) neg. (saline)
		2. concen'ted by method (A)	Frog (male)	neg.	
		3. concen'ted by method (B)**	Friedman Asch.-Zond.	neg. neg.	

\* method (A), Cutler

\*\* method (B), Bradbury *et al*

In this experiment, the time of conception was judged by the following observations. The female began to refuse the male, when he was going to mount her starting in May 1963. She became more gentle and quiet. She never bathed in a water pool in the zoo since the later part of May 1963, although she had previously been very fond of it, even in the cold winter season. The body weight of the fetus, 133.3 kg, was above the average, 70 to 122 kg, of newborn Indian elephants in zoos or circuses. So conception might have occurred a little earlier than April 1963.

From the information reported here we cannot determine the nature and time of excretion of a possible gondotrophin in the urine of elephants in pregnancy, and we have been unable to find another pregnant elephant for further study. Although it may be presumptuous to speculate from one case on the nature of this gonadotrophin in the urine of a pregnant elephant, from the results obtained in the Friedman test and Aschheim-Zondek test, it seems more like FSH than LH in its activity.

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## EXPLANATION OF PLATES

## PLATE I

FIG. 1: "Mating behavior," incomplete copulation — June, 1962.

FIG. 2: Collection of urine by a ladle.

## PLATE II

FIG. 3: Parturition and a stillborn calf.

FIG. 4: "Positive" Friedman reaction on an ovary of a test rabbit. (ca. x 5)



FIG. 1



FIG. 2

GONADOTROPHIN IN THE URINE OF A PREGNANT ELEPHANT



FIG. 3



FIG. 4

GONADOTROPHIN IN THE URINE OF A PREGNANT ELEPHANT